Journal of Medical Virology 61:155–158 (2000)

Persistent Fetal Rubella Vaccine Virus Infection Following Inadvertent Vaccination During Early Pregnancy

J. Hofmann,[1] M. Kortung,[1] B. Pustowoit,[1] R. Faber,[2] U. Piskazeck,[3] and U.G. Liebert[1]*

[1]*Institut für Virologie, Universität Leipzig, Germany*
[2]*Universitätsfrauenklinik, Universität Leipzig, Germany*
[3]*Abteilung für Gynäkologie und Geburtshilfe, Krankenhaus Grimma, Germany*

Inadvertent immunisation of seronegative women with RA27/3 rubella virus live-attenuated vaccine several weeks before and after conception is described. Whereas in 5 cases the vaccine virus was not transmitted vertically, in 1 case vaccination led to the development of persistent fetal infection with prolonged virus shedding for more than 8 months. Sequence analysis carried out on isolates from amniotic fluid, from cord blood leukocytes as well as from infantile urine confirmed an infection by the vaccine strain. At birth, the newborn infant exhibited none of the symptoms compatible with the congenital rubella syndrome and signs indicative for development of late onset disease are not apparent. This observation constitutes the first unequivocal documented case of rubella vaccine virus related to persistent fetal infection. ***J. Med. Virol. 61:155–158, 2000.***

KEY WORDS: Rubella virus; vaccination; pregnancy; sequence analysis

INTRODUCTION

Rubella virus (RV) infection is of considerable concern when a pregnant woman is infected within the first trimester of gestation because in up to 50% of cases the infection leads to severe congenital malformations [Wolinsky, 1996]. Consistent mass immunisation has reduced the incidence of congenital rubella syndrome to less than 10 cases per 100,000 live-births per year [Frey et al., 1998]. World wide the rubella vaccine used most commonly is the live-attenuated vaccine strain RA27/3. Vaccination is highly efficient and large-scale rubella epidemics have been eradicated in immunised populations in recent years. Individual RV infections still occur among adults in childbearing age, however, and cases of the congenital rubella syndrome continue to be reported [Robinson et al., 1994; Zgorniak-Nowosielska et al., 1996]. Furthermore, active immunisation of susceptible pregnant women includes the risk of transmission of the rubella vaccine virus across the placental barrier. In follow-up studies comprising 312 infants delivered of women who were vaccinated against rubella during pregnancy, an association with congenital rubella syndrome was not detected [Bart et al., 1985; Enders, 1985], and the calculated theoretical risk of birth defects does not differ from the background risk of congenital defect in the absence of infection. Nevertheless, guidelines continue to state that precautions should be taken to prevent periconceptional vaccination.

A well-documented case of persistent fetal rubella vaccine virus infection without development of any discernible adverse effect is described after inadvertent vaccination during early pregnancy, whereas in 5 further cases transmission of the vaccine strain of virus was not observed.

MATERIALS AND METHODS

Patient Data

A 25-year-old female (TCW), gravida 1, para 0, attended for in vitro fertilisation had been vaccinated with Rubella Vaccine Live BP Wistar RA27/3 strain (Welcome). At that time she was unaware of a natural spontaneous conception that had occurred 3 weeks previously. The initial serological test for rubella virus antibodies was determined three weeks post-vaccination, when positive IgM and negative IgG levels were detected, consistent with early post-vaccination seroconversion. To test for potential vertical transmission of the vaccine rubella virus an amniocentesis was carried out in Week 16 of gestation (13 weeks post-immunisation). Virus isolation and PCR in amniotic fluid was positive for rubella virus. The patient was referred to the University department of gynaecology and obstetrics, and after repeated prenatal monitoring including cordocentesis fetal rubella virus infection was confirmed. Multiple ultrasound investigations (at gesta-

*Correspondence to: U.G. Liebert, Institut für Virologie, Liebigstrasse 24, 04103 Leipzig. E-mail: liebert@medizin.uni-leipzig.de

Accepted 3 November 1999

tional Weeks 18, 23, 26, 29, 32 and 36) revealed no pathological findings, particularly of the placenta, fetal brain, liver and spleen as well as perfusion of umbilical and fetal cerebral arteries and thoracic aorta were inconspicuous at any time. No complications occurred during pregnancy and in Week 40 of gestation (37 weeks after rubella vaccination) a healthy boy weighing 3450 g was delivered by Caesarean section. Apgar scores were 9/9/10 and NSA-pH 7.34. During physical examination no anomaly was detected and particularly hearing tests on both ears were inconspicuous. To date (age 14 months) the boy has developed regularly and passed the developmental milestones appropriately for his age. He never had a rash, persistent diarrhoea or pneumonitis.

Antibody Tests

Anti-rubella IgG and IgM were detected using commercial assays (Cobas Core, Roche, Basel). The avidity of anti rubella IgG antibodies was determined as the ratio of specific IgG binding in the presence and absence of 4 M urea/hydrogen peroxide in the washing fluid, as described previously [Pustowoit and Liebert, 1998]). Rubella virus like particles were used for Western blot analysis as described [Hobman et al., 1994; Pustowoit and Liebert, 1998].

Virus Isolation, RT-PCR and Sequencing

Amniotic fluid or urine were incubated at 35°C on confluent Vero 76 cell cultures. After 5 to 6 days, the supernatant was passaged on naive Vero cells, supernatant of mock-infected cells served as negative control. After the second passage virus-induced cytopathology was observed as described [Hofmann et al., 1999].

The expression of viral proteins in infected cells was demonstrated by indirect immunofluorescence using a goat polyclonal antibody to RV (Advanced Biotechnologies Inc.).

Total RNA was extracted from cell cultures or various maternal as well as fetal material using the RNAclean kit (AGS, Heidelberg) and RT reactions were carried out following standard procedures. For cDNA preparation the anti-sense oligonucleotide RA6 (5′-AGTGGTGTGTGTGCCATACACA, complementary to nucleotides 9540–9518 within the E1 region of the RV genome) was used. For amplification of putative rubella nucleic acid a single round PCR was performed using the primers R2/R7 [Bosma et al., 1995]. To confirm the specificity of the resulting amplicons, the biotinylated primer R8C served as gene probe in a PCR enzyme linked oligo sorbent assay (Boehringer Mannheim, Penzberg). Plasmid DNA containing the open reading frame coding for the RV structural proteins [Hofmann et al., 1999] served as positive control.

The viral isolates were characterised by nucleotide sequencing using the kit "Big Dye Terminator Cycle Sequencing" (PE Applied Biosystems). The PCR was performed on target cDNA, prepared with the oligonucleotide RA6, with combinations of the oligonucleotides RA1–RA5 (20 pmol each). The sequence of RA1 through RA5 primers, all located within the E1-gene, is RA1: 5′-GAGGAG-GCTTTCACCTACCTCT (nucleotides 8252–8274), RA2: 5′-ACGCGCGCTGTGTG-CCATT (nucleotides 8497–8478), RA3: 5′-GACACC-GTGATGAGCGTGTT (nucleotides 8657–8677), RA4: 5′-TCAGGGGAATGGCGTTGGCAA (nucleotides 8974–8953) and RA5: 5′-CCCGGTCACACGCACATTGC (nucleotides 9277–9296 identical to R14C) [Bosma et al. 1995]). The cDNA was denatured at 95°C for 5 min, followed by 40 repetitive cycles of 55°C/30 sec for annealing, 72°C/2 min for primer extension, and 95°C/30 sec for denaturation. After 15 min at 72°C final extension samples were cooled down and precipitated. 100 ng of precipitated DNA's were used for the sequence reaction according to the manufacturer's instructions. After repeated ethanol precipitation the mix was analysed in an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems).

RESULTS

The antibody tests of maternal serum samples illustrate development of post-vaccination rubella virus-specific humoral immune response (Table I) with rising IgG titers (>500 IU/ml) and consistently maturing avidity. Five months after immunisation antibodies to all rubella virus structural proteins including glycoprotein E2 were detectable, indicating that a resilient protective immunity had developed. In contrast to maternal IgM levels, fetal IgM remained high for the entire gestation. On many occasions rubella virus, referred to as RV-TCW, was isolated from fetal blood and amniotic fluid. Rubella virus RNA was amplified directly from fetal material (data not shown). The specificity of the PCR products was shown by hybridisation with rubella E1 gene-specific probe. The excretion of rubella virus in the infant's urine was also shown by RT-PCR, isolation of rubella virus in cell culture was confirmed by indirect immunofluorescence as well as by PCR. In contrast to the positive results obtained from fetal material, rubella virus genome could not be detected in maternal blood mononuclear cells obtained at the same time.

The nucleotide sequences of PCR products from the viral RNA obtained directly from amniotic fluid, cord blood mononuclear cells and urine as well as RV-TCW isolated from cell culture were determined. In total 985 nucleotides that represent 70% of the rubella virus-E1 gene were analysed by forward and reverse sequencing and compared with a consensus [Frey and Abernathy, 1993]. The sequences of all isolates from fetal material were identical and shared with RA27/3 vaccine the characteristic nucleotide exchanges at positions 8768, 8879, 8880 and 8996 [Pugachev et al., 1997]. Additionally, the nucleotides at position 8301 (R = G or A) and 8594 (Y = C or T) found in the isolates were identical to those of RA27/3. Four of the 6 mutations detected were silent. The mutation at nucleotide position 8880 lead to an amino acid change from Tyr (consensus) to His (RA27/3 and RV-TCW), and that at nt 8301 from

TABLE I. Serological and Virological Findings in Various Maternal and Fetal/Infant Samples

Weeks post vaccination	Week of gestation	Material	IgG (IU/ml)	IgM (index)	IgG-avidity (index)	Virus isolation	RT-PCR
Patient (mother)							
3	6	serum	<10	4.6	NA	ND	ND
19	16	serum	361	1.2	0.62	ND	ND
24	27	serum	282	1.0	0.74	ND[a]	neg[a]
36	39	serum	>500	neg	0.81	ND[a]	neg[a]
Fetus/infant							
13	16	amniotic fluid	NA	NA	NA	pos	pos
19	22	cord blood	340	30	ND	ND	ND
36	39	cord blood	>500	33	ND	pos[a]	pos[a]
37	40[b]	serum	>500	26	0.70	ND	ND
		urine	NA	NA	NA	pos	pos
60	[c]	serum	66	neg	0.28	ND[a]	neg[a]
		urine	NA	NA	NA	neg	neg
93	[d]	serum	12	ng	0.18	ND[a]	neg[a]
		urine	NA	NA	NA	neg	neg

[a]PCR/virus isolation done in peripheral blood mononuclear cells.
[b]At birth.
[c]23 weeks of age.
[d]14 months of age.
NA, not applicable; ND, not done.

Thr (consensus) to Ala (RA27/3 and RV-TCW), respectively.

Five additional cases of pre- or periconceptional rubella vaccination were observed during the last two years. All the women developed characteristic post-vaccination seroconversion but no evidence was obtained for fetal infections by serological and molecular methods in all available specimens. Furthermore, replicating virus could not be recovered from the infantile urine or throat swabs of these 5 newborns.

DISCUSSION

This report describes accidental periconceptional rubella vaccination in 6 seronegative women. In one case persistence of fetal anti-rubella IgM and constant virus shedding over a period of more than 8 months were consistent with the development of long-term persistent fetal infection as a consequence of immunisation with the vaccine strain RA27/3. The outcome of the intrauterine infection was benign, no defects were found in the baby and virus shedding ceased by age of 5 months. The apparent failure of the infant to synthesise IgG by 14 months post partum might be a result of either inadequate maturation of the anti-rubella immune response or of at least partial immune tolerance against rubella virus with lack of Ig-class switch from M to G. The case illustrates, that despite reduced virulence of live attenuated rubella vaccine vertical transmission from mother to fetus is not prevented. The infection with the vaccine virus as proved by nucleotide sequence analysis did not result in a congenital defect and there is no indication for the development of late onset disease as frequently observed in fetal infections with wild type virus.

As long as life virus vaccines are used widely potential adverse effects cannot be completely prevented from occurring. The problems associated with intrauterine infection include the unknown risk of developing late onset symptoms such as juvenile diabetes mellitus type 1, that may be the consequence of virus mediated lysis of insulin producing beta cells on a background of genetic susceptibility to autoimmunity [Freij et al., 1988]. Using infectious viral cDNA instead of live-attenuated vaccines might provide an opportunity to circumvent such risks. A potential prototype of a non-replicating rubella virus vaccine derived from infectious cDNA has been shown recently to induce in mice the generation of a humoral immune response directed against glycosylated envelope proteins [Pugatcheva et al., 1999]. So far no viral sequences, however, have been identified that might be responsible for unwelcome complications of rubella vaccination. Moreover, the availability of an animal model would be required to shed light on these questions and may provide solutions for some of the problems mentioned.

Previous reports described only a few cases of successful isolation of rubella virus from fetal tissue obtained after abortion [Banatvala et al., 1981; Bernstein and Ogra, 1980]. Due to the lack of available appropriate technologies, however, no proof could be presented that it was the vaccine virus that had been isolated.

Phylogenetic analysis revealed that the sequence of rubella virus genomes is remarkably well conserved among different isolated and vaccine strains [Frey et al., 1998]. In the context of observations that multiple passages of rubella virus in cell culture do not result necessarily in accumulation of mutations [Bosma et al., 1996] it is not surprisingly that in the present case all RV-TCW isolates were identical to the original vaccine sequence. There are only 6 nucleotide changes between the consensus sequence on the one side and RA27/3 or RV-TCW on the other side.

The nucleotide change at position 8880 in RV-TCW as well as RA27/3 that results in an amino acid change at position 2960 from Tyr to His is of particular interest. This amino acid is near one of 3 N glycosylation

sites within the E1 protein that might play a role in the pathogenesis. Because X-ray crystal structure analysis of E1 protein is not available, structural as well as functional consequences of the amino acid change are not known. Furthermore, the potential virologic relevance remains as yet speculative.

With the availability of appropriate technology, it is now possible to determine the risk of infectious agents for pregnancy much more readily than in previous decades. In the case of rubella virus the most logical and successful way consists in investigation of amniotic fluid. This material is available as early as 10 weeks of gestation, and therefore several weeks before cord blood. Attempts to isolate rubella virus on cell culture should be undertaken, followed by PCR on RNA extracted from these cells, whether or not cytopathic effects can be observed in cell cultures [Eggerding et al., 1991; Ho-Terry et al., 1990]. Whenever inadvertent immunisation occurs during pregnancy, attempts to isolate the virus from amniotic fluid or fetal cord blood followed by nucleotide sequence analysis should be performed as the only way to distinguish intercurrent wild type from vaccine virus infection.

ACKNOWLEDGMENTS

We gratefully acknowledged Mrs. K. Heise for help with sequence analysis. We wish to thank Prof. W. Raue and Dr. P. Foley for critically reading the manuscript and helpful discussions.

REFERENCES

Banatvala JE, O'Shea S, Best JM, Nicholls MV, Cooper K. 1981. Transmission of RA27/3 rubella vaccine strain to products of conception. Lancet 1:392.

Bart SW, Stetler HC, Preblud SR, Williams NM, Orenstein WA, Bart KJ, Hinman AR, Herrmann KL. 1985. Fetal risk associated with rubella vaccine: an update. Rev Infect Dis 7(Suppl):95–102.

Bernstein DI, Ogra PL. 1980. Fetomaternal aspects of immunisation with RA27/3 live attenuated rubella virus vaccine during pregnancy. J Pediatr 97:467–470.

Bosma TJ, Best JM, Corbett KM, Banatvala JE, Starkey WG. 1996. Nucleotide sequence analysis of a major antigenic domain of the E1 glycoprotein of 22 rubella virus isolates. J Gen Virol 77:2523–2530.

Bosma TJ, Corbett KM, O'Shea S, Banatvala JE, Best JM. 1995. PCR for detection of rubella virus RNA in clinical samples. J Clin Microbiol 33:1075–1079.

Eggerding FA, Peters J, Lee RK, Inderlied CB. 1991. Detection of rubella virus gene sequences by enzymatic amplification and direct sequencing of amplified DNA. J Clin Microbiol 29:945–952.

Enders G. 1985. Rubella antibody titers in vaccinated and nonvaccinated women and results of vaccination during pregnancy. Rev Infect Dis 7:S103–S107

Freij BJ, South MA, Sever JL. 1988. Maternal rubella and the congenital rubella syndrome. Clin Perinatol 15:247–257.

Frey TK, Abernathy ES. 1993. Identification of strain-specific nucleotide sequences in the RA 27/3 rubella virus vaccine. J Infect Dis 168:854–864.

Frey TK, Abernathy ES, Bosma TJ, Starkey WG, Corbett KM, Best JM, Katow S, Weaver SC. 1998. Molecular analysis of rubella virus epidemiology across three continents, North America, Europe, and Asia, 1961–97. J Infect Dis 178:642–650.

Ho-Terry L, Terry GM, Londesborough P. 1990. Diagnosis of fetal rubella virus infection by polymerase chain reaction. J Gen Virol 71:1607–1611.

Hobman TC, Lundstrom ML, Mauracher CA, Woodward L, Gillam S, Farquhar MG. 1994. Assembly of rubella virus structural proteins into virus-like particles in transfected cells. Virology 202:574–585.

Hofmann J, Pletz MWR, Liebert UG. 1999. Rubella virus-induced cytopathic effect in vitro is caused by apoptosis. J Gen Virol 80: 1657–1664.

Pugachev KV, Abernathy ES, Frey TK. 1997. Genomic sequence of the RA27/3 vaccine strain of rubella virus. Arch Virol 142:1165–1180.

Pugatcheva SO, Abernathy ES, Vzorov AN, Compans RW, Frey TK. 1999. Development of a rubella virus DNA vaccine. Vaccine 17: 2104–2112.

Pustowoit B, Liebert UG. 1998. Predictive value of serological tests in rubella virus infection during pregnancy. Intervirology 41:170–177.

Robinson J, Lemay M, Vaudry WL. 1994. Congenital rubella after anticipated maternal immunity: two cases and a review of the literature. Pediatr Infect Dis J 13:812–815.

Wolinsky, J. 1996. Rubella. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B, editors. Virology. New York: Raven Press. pp 815–838.

Zgorniak-Nowosielska I, Zawilinska B, Szostek S. 1996. Rubella infection during pregnancy in the 1985–86 epidemic: follow-up after seven years. Eur J Epidemiol 12:303–308.